

- (c) separating said multipotent stem cells from said cells that attach to said culture substrate; and
- (d) repeating steps (b) and (c) until at least 30% of the cells are multipotent stem cells which are self renewing, form non-adherent clusters, express nestin, and can differentiate into neuronal and mesodermal cell types, or progeny of said multipotent stem cells.

### **REMARKS**

Claims 1-46 constitute the pending claims in the present application. Applicants add new claims 43-46. Support for the subject matter of these claims is found throughout the specification. No new matter has been entered. Applicants respectfully request reconsideration in view of the following remarks. Issues raised by the Examiner will be addressed below in the order they appear in the prior Office Action.

#### ***Formal Matters***

Applicants note that new claims 35-42 were added in Paper No. 9. However, these claims have been withdrawn from consideration as allegedly directed to a non-elected invention. Applicants respectfully disagree, and contend that new claims 35-42 can be considered in the present application. Specifically, Applicants direct the Examiner's attention to claim 35. Applicants maintain that claim 35 is directed to methods of producing a population of multipotent stem cells. Such subject matter is the same as that of claims 18-20 (the elected invention). Accordingly, Applicants request that claims 18-20 and 35 be considered in the present application.

#### ***35 U.S.C. 112, second paragraph***

Claims 18-20 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for allegedly failing to particularly point out and distinctly claim the subject matter which applicants

regard as the invention. Applicants traverse this rejection to the extent that it is maintained in light of the amended claims.

a. The claims are rejected because one of skill in the art is allegedly unable to ascertain the metes and bounds of the term “substantially”. Applicants respectfully disagree. The term substantially is defined in the specification (page 9, line 24 – page 10, line 1). Furthermore, the term is used throughout the disclosure to describe the cells isolated by the methods of the present invention (see, for example, pages 5-6). Accordingly, one of skill in the art is given not only a mere definition, but also multiple contexts in which to appreciate the meaning of the metes and bounds of the term “substantially.”

The Office Action alleges that the term is a relative term, and that the relative nature of the term renders the claims indefinite. However, Applicants respectfully disagree with this reasoning, and in fact contend that a relative term is necessary to properly describe the claimed subject matter. The invention is directed to methods of obtaining substantially purified populations of cells with particular characteristics. An important feature of the invention is the percentage of the desired stem cells in the culture in comparison to other non-stem cell types. This description requires a relative term. In this case, the use of an absolute term would be meaningless and would fail to particularly describe the invention.

Applicants contend that the term substantially is defined in the specification, and accordingly one of skill in the art can readily appreciate the metes and bounds of the claimed subject matter. Furthermore, Applicants contend that the very nature of the invention demands the use of a relative term in order to particularly point out and distinctly claim Applicants’ invention. Reconsideration and withdrawal of the rejection is respectfully requested.

### ***35 U.S.C. 102(a)***

Claims 18-20 are rejected under 35 U.S.C. 102(a) as allegedly being anticipated by Sosnowski et al. Applicants traverse this rejection to the extent that it is maintained in light of the amended claims.

In accordance with MPEP 2131 and with the Courts, “a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.” *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). “The identical invention must be shown in as complete detail as is contained in the...claim.” *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

Sosnowski et al. fail to satisfy the criteria clearly delineated by the MPEP and the Courts for anticipating the claimed invention. Applicants’ claims are directed to a method of producing a purified population of multipotent stem cells which are self renewing, form non-adherent clusters, and express nestin. The techniques employed by Sosnowski et al. fail to meet each and every limitation of the pending claims. Most notably, the methods of Sosnowski et al. fail to produce a population of cells which are self renewing, and fail to produce a population of cells which can differentiate into both neuronal and mesodermal cell types, as required by the pending claims. The cells of Sosnowski et al. spontaneously differentiate in culture, and thus can certainly not be considered a self renewing population of cells. That the cells of Sosnowski et al. differentiate to neuronal and glia cells is indicative of just that: the ability of the cells to differentiate to form neurons and glia. This characteristic neither demonstrates nor suggests that the cells are a self renewing population of cells.

The teachings of Sosnowski et al. fail to delineate each and every limitation of the pending claims. Accordingly, the teachings of Sosnowski et al. fail to satisfy the standards required to anticipate the claimed subject matter. Reconsideration and withdrawal of this rejection are respectfully requested.

### ***35 U.S.C. 102(b)***

Claims 18-20 are rejected under 35 U.S.C. 102(b) as allegedly being anticipated by Ronnett et al. Applicants traverse this rejection to the extent that it is maintained in light of the amended claims.

Both the methods described by Ronnett et al. and the presently claimed methods begin with tissue such as olfactory epithelium. However, this is where the similarities between Applicants' invention and the cited reference end. Applicants' invention is specifically directed to methods of purifying multipotent stem cells which are characterized by specific properties. In contrast, the cells purified by the methods of Ronnett are not stem cells. The methods of Ronnett et al. teach the isolation of "olfactory neurons which retain their excitability in response to odorants." (column 1, lines 67-68). The characteristics used to describe these cells are not only inconsistent with the characteristics of Applicants' multipotent stem cells, but are in fact inconsistent with the characteristics of any stem cell population. For example, the neurons of Ronnett et al. demonstrate "responsiveness to physiologic levels of odorants, said neurons expressing vimentin, olfactory marker protein and neuron-specific enolase." (column 2, lines 47-49). To further illustrate the inconsistencies of these characteristics with any stem cell population, Applicants enclose herewith the abstract from a recent publication from Ronnett himself which supports Applicants' position that the cells disclosed by Ronnett et al. are not stem cells and the methods disclosed are not methods of isolating stem cells (Moon et al. (2002); attached hereto as Exhibit A). Specifically, Exhibit A identifies olfactory marker protein as a marker of mature olfactory receptor neurons (ORNs) (Exhibit A, lines 10-11). The teachings of Ronnett et al., as supported by the teachings of Exhibit A, are directed to methods of isolating and culturing a mature, non-stem cell, neuronal population. These teachings in no way anticipate the isolation and culture of a completely different cell type, as provided by Applicants' invention.

Clearly, the olfactory neurons described by Ronnett et al. are not stem cells, and thus the isolation and culture of such cells in no way anticipates the claimed invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

### CONCLUSION

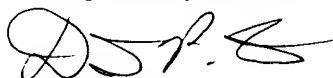
In view of the foregoing amendments and remarks, Applicants submit that the pending claims are in condition for allowance. Early and favorable reconsideration is respectfully solicited. The Examiner may address any questions raised by this submission to the undersigned

at 617-951-7000. Should an extension of time be required, Applicants hereby petition for same and request that the extension fee and any other fee required for timely consideration of this submission be charged to **Deposit Account No. 18-1945**.

Date: September 6, 2002

*Customer No: 28120*  
Docketing Specialist  
Ropes & Gray  
One International Place  
Boston, MA 02110  
Phone: 617-951-7000  
Fax: 617-951-7050

Respectfully Submitted,



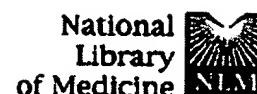
---

David P. Halstead  
Reg. No. 44,735



Xh.b. A

PubMed



PubMed Nucleotide Protein Genome Structure PopSet Taxonomy OMIM Bc  
Search PubMed for Go Clear  
Limits Preview/Index History Clipboard Details

About Entrez

Display Abstract Sort Save Text Clip Add Order

Text Version

Entrez PubMed

Overview  
Help | FAQ  
Tutorial  
New/Noteworthy  
E-Utilities

PubMed Services

Journal Browser  
MeSH Browser  
Single Citation Matcher  
Batch Citation Matcher  
Clinical Queries  
LinkOut  
Cubby

Related Resources

Order Documents  
NLM Gateway  
TOXNET  
Consumer Health  
Clinical Alerts  
ClinicalTrials.gov  
PubMed Central

Privacy Policy

1: Proc Natl Acad Sci U S A 2002 Jun 25;99(13):9015-20

Related Articles, NEW Links

Full text article at  
[www.pnas.org](http://www.pnas.org)

## Leukemia inhibitory factor inhibits neuronal terminal differentiation through STAT3 activation.

Moon C, Yoo JY, Matarazzo V, Sung YK, Kim EJ, Ronnett GV.

Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

The discovery of stem cells in the adult central nervous system raises questions concerning the neurotrophic factors that regulate postnatal neuronal development. Olfactory receptor neurons (ORNs) are a useful model, because they are capable of robust neurogenesis throughout adulthood. We have investigated the role of leukemia inhibitory factor (LIF) in postnatal neuronal development by using ORNs as a model. LIF is a multifunctional cytokine implicated in various aspects of neuronal development, including phenotype determination, survival, and in response to nerve injury. LIF-deficient mice display significant increases, both in the absolute amount and in the number of cells expressing olfactory marker protein, a marker of mature ORNs. The maturation of ORNs was significantly inhibited by LIF in vitro. LIF activated the STAT3 pathway in ORNs, and transfection of ORNs with a dominant negative form of STAT3 abolished the effect of LIF. These findings demonstrate that LIF negatively regulates ORN maturation via the STAT3 pathway. Thus, LIF plays a critical role in controlling the transition of ORNs to maturity. Consequently, a population of ORNs is maintained in an immature state to facilitate the rapid repopulation of the olfactory epithelium with mature neurons during normal cell turnover or after injury.

PMID: 12084939 [PubMed - indexed for MEDLINE]

Display Abstract Sort Save Text Clip Add Order

Write to the Help Desk  
NCBI | NLM | NIH